

Assays Using Fluorescent Polarization

Reference	Bolger et al. (1998)	Hanioka et al. (1999)	Hashimoto et al. (2000)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER	human ER	human ER
<i>Source of receptor</i>	PanVera	n.p.	n.p.
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant	recombinant
<i>Buffer for assay of receptor</i>	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin
<i>Protein concentration</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Ligand used</i>	ES2	ES2	ES2
<i>Concentration of estrogen</i>	1 nM	1 nM	1 nM
<i>Fluorescent ligand</i>	FES1 ER 13 nM, ER 10 nM	FES1 ER 13 nM, ER 10 nM	FES1 ER 13 nM, ER 10 nM
<i>Concentration of fluorescent ligand</i>	2 nM	2 nM	2 nM
<i>Solvent used to dissolve competing ligand</i>	10 mM ethanol	10 mM ethanol	10 mM ethanol
<i>Concentration range of competing ligand</i>	200 µM	200 µM	200 µM
<i>Number of replicates</i>	3	3	3
<i>Number of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	60 min	60 min	60 min
<i>Temperature of incubation</i>	room temp	room temp	room temp
Data calculations			
<i>Fluorescence anisotropy</i>	490 nm excitation; 530 nm emission filter	360 nm excitation; 535 nm emission filter	360 nm excitation; 530 nm emission filter
<i>Program or method used for calculating data</i>	Anisotropy converted to fraction bound	Nonlinear least squares regression	Anisotropy converted to percent inhibition
<i>Data plotted as</i>	Ligand bound=fraction bound x ligand conc.	Millipolarization vs. conc. of chemicals	Percent inhibition vs. competitor conc.
<i>Data format in paper (e.g., IC₅₀, K_d)</i>	K _d	IC ₅₀	Percent inhibition
<i>Calculation of RBA</i>	Nonlinear least squares regression	Nonlinear least squares regression	n.a.

Abbreviations: n.p. = not provided;
n.a. = not applicable; RBA = relative binding affinity

Assays Using Fluorescent Polarization

Reference	Nikov et al. (2000)	Nikov et al. (2001)	Parker et al. (2000)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER and ER	human ER and ER	human ER and ER
<i>Source of receptor</i>	PanVera	PanVera	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant	n.p.
<i>Buffer for assay of receptor</i>	100 mM KaPO ₄ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide	100 mM KaPO ₄ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide	100 mM KaPO ₄ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide
<i>Protein concentration</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Ligand used</i>	ES2	ES2	ES2
<i>Concentration of estrogen</i>	1 nM	1 nM	1 nM
<i>Fluorescent ligand</i>	FES1	FES1	FES1 ER 13 nM, ER 10 nM
<i>Concentration of fluorescent ligand</i>	n.p.	n.p.	n.p.
<i>Solvent used to dissolve competing ligand</i>	8 mM ethanol	8 mM ethanol	n.p.
<i>Concentration range of competing ligand</i>	n.p.	n.p.	n.p.
<i>Number of replicates</i>	n.p.	n.p.	n.p.
<i>Number of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	60 min	60 min	2 hours
<i>Temperature of incubation</i>	room temp	room temp	room temp
Data calculations			
<i>Fluorescence anisotropy</i>	490 nm excitation; 530 nm emission filter	490 nm excitation; 530 nm emission filter	483 nm excitation; 536 nm emission filter
<i>Program or method used for calculating data</i>	Nonlinear least squares regression, Prism, Graphpad (San Diego, CA)	Nonlinear binding isotherm	Nonlinear least squares regression, Prism, Graphpad (San Diego, CA)
<i>Data plotted as</i>	Percent inhibition vs. competitor conc.	Percent inhibition vs. competitor conc.	Millipolarization vs. conc. of chemicals
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	IC ₅₀	IC ₅₀
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ ligand X100	IC ₅₀ E ₂ /IC ₅₀ ligand X100	IC ₅₀ E ₂ /IC ₅₀ ligand X100

Abbreviations: n.p. = not provided;
 n.a. = not applicable; RBA = relative binding affinity

Assays Using Fluorescent Polarization

Reference	Saito et al. (2000)
Preparation of receptor	
<i>Species and subtype of receptor</i>	human ER
<i>Source of receptor</i>	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant
<i>Buffer for assay of receptor</i>	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin
<i>Protein concentration</i>	n.p.
Competitive binding assay	
<i>Ligand used</i>	ES2
<i>Concentration of estrogen</i>	1 nM
<i>Fluorescent ligand</i>	FES1 ER 13 nM, ER 10 nM
<i>Concentration of fluorescent ligand</i>	2 nM
<i>Solvent used to dissolve competing ligand</i>	10 mM ethanol
<i>Concentration range of competing ligand</i>	10 nM -10 µM
<i>Number of replicates</i>	3
<i>Number of times assay repeated</i>	n.p.
<i>Time of incubation</i>	60 min
<i>Temperature of incubation</i>	room temp
Data calculations	
<i>Fluorescence anisotropy</i>	490 nm excitation; 530 nm emission filter
<i>Program or method used for calculating data</i>	n.p.
<i>Data plotted as</i>	Percent inhibition vs. competitor conc.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	n.p.
<i>Calculation of RBA</i>	n.p.

Abbreviations: n.p. = not provided;
 n.a. = not applicable; RBA = relative binding affinity